

# Proximate Causes of Infertility and Embryo Mortality in Captive Zebra Finches

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**ABSTRACT:** Some species show high rates of reproductive failure, which is puzzling because natural selection works against such failure in every generation. Hatching failure is common in both captive and wild zebra finches (*Taeniopygia guttata*), yet little is known about its proximate causes. Here we analyze data on reproductive performance (the fate of >23,000 eggs) based on up to 14 years of breeding of four captive zebra finch populations. We find that virtually all aspects of reproductive performance are negatively affected by inbreeding (mean  $r = -0.117$ ); by an early-starting, age-related decline (mean  $r = -0.132$ ); and by poor early-life nutrition (mean  $r = -0.058$ ). However, these effects together explain only about 3% of the variance in infertility, offspring mortality, fecundity, and fitness. In contrast, individual repeatability of different fitness components varied between 15% and 50%. As expected, we found relatively low heritability in fitness components (median: 7% of phenotypic variation and 29% of individually repeatable variation). Yet some of the heritable variation in fitness appears to be maintained by antagonistic pleiotropy (negative genetic correlations) between male fitness traits and female and offspring fitness traits. The large amount of unexplained variation suggests a potentially important role of local dominance and epistasis, including the possibility of segregating genetic incompatibilities.

**Keywords:** inbreeding, senescence, early nutrition, reproductive failure, quantitative genetics, sexual antagonism.

## Introduction

Reproductive performance, including offspring survival, is subject to strong directional selection in every genera-

tion. Such strong selection works not only on individuals that live in their natural habitat but also on those that live in captivity, unless artificial selection counters it. Thus, it is puzzling that some populations (or species) have substantial difficulties with successful reproduction, shown as high rates of infertility or embryo mortality. Prominent examples of frequent reproductive failure include humans (De Braekeleer and Dao 1991; Sierra and Stephenson 2006; Miyamoto et al. 2012) and other animals both in natural environments (Lyon 1986; Grossen et al. 2012) and in captive conditions (Ayalon 1978; Bunin et al. 2008; Gwaza et al. 2016; Griffith et al. 2017). Given that selection constantly removes genetic variants that lead to poor performance, one might suspect that reproductive failure typically results from inbreeding (Briskie and MacIntosh 2004), because selection against recessive deleterious mutations is inefficient, or from environmental factors (Jurewicz et al. 2009), such as pollutants (Jackson et al. 2011). However, as explained below, the range of possible explanations is much wider.

Reproductive failure and individual survival are complex traits and hence may be influenced by multiple genetic components that can be evolutionarily stable. For instance, reproductive failure and mortality may be caused by selfish genetic elements that are self-promoting at the cost of organismal fitness (Sandler et al. 1959; Lyon 1986; Safronova and Chubykin 2013; Lindholm et al. 2016). Additive genetic variants can also be preserved under intralocus sexual antagonism, where genes that are beneficial to one sex impose detrimental effects on the other (Foerster et al. 2007; Van Doorn 2009; Innocenti and Morrow 2010). Furthermore, there might be evolutionary trade-offs between traits, such that individuals that invest more in reproduction might show lower survival rates (Stearns 1989; Schluter et al. 1991). A few recent genetic and genomic studies detected genetic variants (e.g., specific genes) involved in dominance effects or rare variants that show main effects

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on reproductive traits (e.g., Christians et al. 2000; Safronova and Chubykin 2013; Kim et al. 2017; Knief et al. 2017). As an extreme example, a balanced system of two nonrecombining lethal alleles was identified in crested newts *Triturus cristatus*, where all embryos that are homozygous for chromosome 1 (about 50% of all embryos) die during development (Sims et al. 1984; Grossen et al. 2012).

Despite the development of new genomic tools, it remains difficult to identify and examine the genetic components that show antagonistic effects or to involve more than one locus, that is, intra- and interlocus genetic incompatibilities (Dobzhansky 1936; Fishman and Willis 2006; Johnson 2008; Eroukhmanoff et al. 2016). This difficulty is likely due to the complexity of interactions between multiple loci and between the genotype and the environment (Carrell and Aston 2011; Krausz and Riera-Escamilla 2018). If animals in captivity show high rates of reproductive failure because they are not adapted to a given artificial environment, selection can act on the standing genetic variance. This would result in a transient phase where fitness is heritable until the population is better able to cope with the new environment (e.g., as a result of behavioral and physiological adaptations to captivity). In general, the genetic basis of reproductive failure and variation in survival remains largely unclear in most species.

The zebra finch is a good model species to study how survival and reproductive performance of the two sexes are correlated at the additive genetic level. The zebra finch is a short-lived songbird that easily breeds in captivity (Zann 1996), and its reproductive performance varies extensively among individuals under controlled breeding conditions in both domesticated and recently wild-derived populations (Griffith et al. 2017; Wang et al. 2017). In the wild, the rate of hatching failure (infertile eggs and dead embryos) was estimated to be >15% (table 1). This excludes clutches that failed completely, because nest desertion cannot be ruled out as the reason of failure. In lab stocks, the average proportion of eggs remaining apparently unfertilized ranged from 17% in aviary breeding to 30%–35% in cage breeding (table 1), while average embryo mortality rates varied between 24% and 75% (table 1). Average nestling mortality rates were also high (table 1). Although some of the variation has been explained by specific treatment effects (e.g., inbreeding, force pairing, maternal stress; Hemmings et al. 2012; Ihle et al. 2015; Khan et al. 2016), the high baseline levels of infertility and embryo and nestling mortality remain largely unexplained.

To better understand this variation in reproductive performance and individual survival, we here report on a comprehensive quantitative genetic analysis of life span, fecundity, infertility, offspring mortality, and other fitness-related traits that cover most phases of reproduction for the two sexes (table 2). We quantified the effects of in-

breeding, age, and an individual's early nutritional condition on all measured aspects of reproductive performance and survival.

Wild zebra finches have a remarkably large effective population size (Balakrishnan and Edwards 2009), where inbreeding is almost completely absent (Knief et al. 2015a). In contrast, in captivity, mating between related individuals is practically inevitable in the long run (Knief et al. 2015a). The level of inbreeding typically correlates negatively with offspring survival, individual fitness, and various morphological and life-history traits (Charlesworth and Charlesworth 1987; Keller and Waller 2002), for instance, in *Drosophila* (Garcia et al. 1994; Bechsgaard et al. 2013; Tan et al. 2013), in wild populations of lizards (Michaelides et al. 2016), and in mammals (Hoffman et al. 2014; Huisman et al. 2016). This is also true for captive zebra finches, whereby the estimated effect sizes of inbreeding depression vary widely among studies (Bolund et al. 2010a; Forstmeier et al. 2012; Hemmings et al. 2012).

Aging, or senescence, typically leads to a decline in reproductive function at old age, for example, in birds (Bouwhuis et al. 2009; Lecomte et al. 2010) and humans (Speroff 1994; Shirasuna and Iwata 2017). In zebra finches breeding in cages, male and female fertility declined when individuals became older (Knief et al. 2017). More generally, the relationship between age and reproductive performance is often quadratic, with an initial increase in performance due to gained experience that may mask any early-starting decline caused by deterioration of the body (Harely 1990; Bouwhuis et al. 2009; Lecomte et al. 2010).

The conditions that an individual experienced during early development may also affect fitness later in life. Such permanent environmental effects have been demonstrated using brood size manipulations, and they may affect individual behavior and reproductive investment (Gorman and Nager 2004; Tschirren et al. 2009; Rickard et al. 2010; Boersma et al. 2014). In zebra finches, being raised in enlarged broods apparently did not affect later performance (Tschirren et al. 2009). However, a nonexperimental measure of individual early-growth condition, namely, body mass measured at 8 days of age (which ranges from 2 to 12 g), had a significant but small effect on fitness later in life (Bolund et al. 2010b).

For this study, we used systematically recorded data on individual body mass at 8 days of age and on reproductive parameters and survival for four captive populations of zebra finches with an error-free pedigree. The aims of this study were (1) to estimate and compare the effect sizes of inbreeding, early nutritional condition, and age on reproductive performance traits; (2) to estimate the relative importance of individual and pair identity (i.e., repeatability) on reproductive performance; (3) to quantify the heritability of individual reproductive performance;

**Table 1:** Summary of rates of hatching failure, infertility, and embryo and offspring mortality reported in the literature on zebra finches

Population	Sample description	Hatching failure (%)	Infertility (%)	Embryo mortality (%)	Nestling mortality (%)	Reference
Wild	1,156 eggs; clutches that produced no nestlings were removed	>17	...	...	...	Zann 1996
Wild	872 eggs; clutches that produced no nestlings were removed	16	...	...	9	Griffith et al. 2008
La Trobe University, Australia, domesticated	31 untreated and 25 CORT-treated pairs; clutches that produced no nestlings and all first eggs were removed	Untreated: 24; treated: 45	Untreated: 7; treated: 15	Untreated: 10; treated: 29	...	Khan et al. 2016
Max Planck Institute for Ornithology, Germany, domesticated (from Sheffield, UK)	11,617 eggs	...	30	...	...	Knief et al. 2015b
Max Planck Institute for Ornithology, Germany, recently wild derived (from Bielefeld, Germany)	852 eggs; aviary	...	17	24	45	Ihle et al. 2015
Sheffield University, UK	161 eggs for infertility; 2,884 eggs for hatching failure and nestling mortality	52	35	...	31	Kim et al. 2017
Sheffield University, UK	1,524 eggs; 77 unrelated and 20 sib-sib pairs	...	Unrelated: 9; sib-sib: 11	Unrelated: 59; sib-sib: 75	Unrelated: 55; sib-sib: 67	Hemmings et al. 2012

Note: For the population from La Trobe University, Australia, in treated pairs females were given a corticosterone (CORT) mix after laying the first egg. The CORT mix was made of 0.5 mg of crystalline corticosterone dissolved by 10  $\mu$ L of ethanol, then mixed with 990  $\mu$ L of peanut oil (Khan et al. 2016). Hatching failure indicates the proportion of eggs that do not hatch. Infertility indicates the proportion of eggs that show no sign of development. Embryo mortality indicates the proportion of fertilized eggs where the embryo died before hatching. Nestling mortality indicates the proportion of nestlings that died before fledging or independence.

and (4) to test whether some of the heritable components can be maintained by antagonistic pleiotropy, by analyzing the additive genetic correlations between reproductive performance traits and life span across the two sexes.

### Methods

Zebra finches are opportunistic breeders that are abundant throughout most of Australia. Individuals become sexually mature around the age of 90 days and then form pairs for life through mutual mate choice. Breeding pairs cooperatively incubate and raise nestlings until they reach independence around the age of 35 days (Zann 1996). Captive zebra finches live for about 4.5 years on average and maximally for 10 years (Zann 1996). The studied zebra finches originated from four populations held at the

Max Planck Institute for Ornithology, Seewiesen, Germany. Details about the population background, rearing conditions, and breeding seasons are provided in the supplemental material (tables S1, S2; tables S1–S11 are available online). Housing in captivity implies that birds are supplied with food ad lib., which is known to maximize their reproductive performance (Lemon and Barth 1992). In brief, we compiled and analyzed up to 14 years of zebra finch reproductive performance data from (1) population Seewiesen, a domesticated population derived from the University of Sheffield with a nine-generation-long error-free pedigree (population 18 in Forstmeier et al. 2007b); (2) population Krakow, a domesticated population that was generated by hybridizing between Krakow (population 11 in Forstmeier et al. 2007b) and Seewiesen populations; (3) population Bielefeld, which was derived from the wild in the late 1980s (population 19 in Forstmeier

**Table 2:** Description of reproductive performance traits in our zebra finch study

Trait	Fixed effects for	Random effects	BLUPs calculated for	Description
	Female			
Clutch size cage	Female	Female	Female	Number of eggs consecutively laid by a single female in a cage (containing one male and one female), allowing for laying gaps of maximally 4 days between subsequent eggs; for 2% (65 of 3,694) clutches that had >7 eggs, they were counted as 7
	...	Male	...	
	...	Pair	...	
Clutch size aviary	Female	Female	Female	Number of eggs consecutively laid by a female in a communal breeding aviary, allowing for laying gaps of maximally 4 days between subsequent eggs; for 5% (173 of 3,663) clutches that had >7 eggs, they were counted as 7
Fecundity aviary	Female	Female	Female	Total number of eggs laid by a female in a communal breeding aviary over the course of a breeding season (35–83 days), where no offspring rearing was allowed
Seasonal recruits	Female	Female	Female	Total number of genetic offspring that survived to independence in a communal breeding aviary, i.e., age 35 days, within a breeding season (83–113 days for egg laying plus about 50 days for rearing)
	Male			
Fertility cage	Female	Female	...	Whether an egg was fertilized by the male in the cage (containing one male and one female)
	Male	Male	Male	
	...	Pair	...	
	Egg	...	...	Whether an egg laid by the social partner of the male in a communal breeding aviary was fertilized by the male (infertile eggs and extrapair fertilizations count as failed within-pair paternity)
Within-pair paternity	Female	Female	...	
	Male	Male	Male	
	...	Pair	...	Total number of eggs fertilized by a male in a communal breeding aviary over the course of a breeding season (35–113 days)
Siring success	Male	Male	Male	
Seasonal recruits	Male	Male	Male	Total number of genetic offspring that survived to independence in a communal breeding aviary, i.e., age 35 days, within a breeding season (83–113 days for egg laying plus about 50 days for rearing)
	Offspring			
Embryo survival	Female	Female	Female	Whether a fertilized egg that was incubated by an individual in a cage (containing one male and one female) or a communal breeding aviary hatched
	Male	Male	...	
	...	Pair	...	
	Embryo	...	...	Whether a nestling that hatched in a cage (containing one male and one female) or a communal breeding aviary survived to independence, i.e., age 35 days
Nestling survival	Female	Female	Female	
	Male	Male	Male	
	...	Pair	...	
	Nestling	...	...	
	Individual			
Life span	Individual	...	Individual	Number of days from the date of hatching to the date of natural death; some missing values were replaced by life expectancy

Note: Traits were measured in the context of either single pairs breeding in a small cage or multiple pairs breeding communally in a large aviary. Fixed effects (focal) are inbreeding coefficient, age, and early condition (mass at day 8). Random effects (focal) are the variance components explained by female, male, or pair identity. Best linear unbiased predictions (BLUPs) are estimated from univariate models where we controlled for significant fixed and random effects. For the offspring trait of embryo survival, female, male, and pair identities refer to the genetic parents of the embryo, whereas for nestling survival, female, male, and pair identities refer to the social parents that raised the nestling. Cage dimensions, before 2012: 60 cm × 40 cm × 45 cm (length × width × height); after 2012: 120 cm × 40 cm × 45 cm. For details of housing conditions, see Bolund et al. (2007). A semioutdoor aviary measured 500 cm × 200 cm × 200 cm (length × width × height).

et al. 2007b); and (4) population Melbourne, which was derived from the wild in the early 2000s (see Jerónimo et al. 2018). All data underlying this study have been deposited in the Open Science Framework (<https://doi.org/10.17605/OSF.IO/TGSZ8>; Pei 2020).

Birds from the two recently wild-derived populations were smaller (~11 g) compared to domesticated birds (~15–16 g, because of selective breeding by aviculturists) and shier, so we bred them only in large semioutdoor aviaries (rather than in small cages; see table 2 for sizes of cage and aviary). Between 2004 and 2017, we bred zebra finches in four settings with various treatments (see tables S1 and S2 for details of breeding seasons): (1) cage breeding, (2) cage laying, (3) aviary breeding, and (4) aviary laying. In cages, single pairs were kept, and hence, partners were assigned. In aviaries, groups of birds were kept together, and individuals could freely form pairs. Group size was typically 12 but ranged from 10 to 42, with sex ratio (proportion of males) ranging from 0.4 to 0.6. In a breeding setup, pairs were allowed to rear their offspring, whereas in a laying setup, all eggs were collected for paternity assignment and replaced by plastic eggs that were removed after 7 or 10 days of incubation. The proportion of individuals that participated in more than one breeding season ranged from 0.23 to 0.84 (mean: 0.47).

In this study, we focus on general effects on reproductive performance in zebra finches, not on population-specific effects. Therefore, in all analyses, we controlled statistically only for between-population differences in reproductive performance (main effects only, no interactions; see below for model details).

#### *Measures of the Focal Fixed Effects: Inbreeding, Age, and Early Nutrition*

We used the pedigree-based inbreeding coefficient  $F_{\text{ped}}$ , calculated using the R package pedigree version 1.4 (Coster 2015), as a measure of the degree of inbreeding of an individual (Wright 1922; Knief et al. 2016b);  $F_{\text{ped}}$  reflects the proportion of an individual's genome that is expected to be identical by descent. Hence,  $F_{\text{ped}}$  can be used to estimate without bias the slope of the regression of fitness over inbreeding (Howrigan et al. 2011; Knief et al. 2016b). For instance, full-sibling mating produces inbred offspring that are expected to have 25% of the genome identical by descent ( $F_{\text{ped}} = 0.25$ ). For practical reasons, all founders were assumed to be unrelated ( $F_{\text{ped}} = 0$ ; Forstmeier et al. 2004), even though their true level of identity by descent is likely about 5% (judging from runs of homozygosity; Knief et al. 2015a).

For all birds, we recorded their exact hatch date. Thus, for models of reproductive performance at the level of

eggs, clutches, and breeding rounds (as the unit of analysis), we used the exact age (in days) of the female or the male when an egg was laid, a clutch started, or a breeding round started, respectively. At the start of reproduction, individuals were 69–2,909 days old (fig. S1; figs. S1–S9 are available online).

On the day of hatching, we individually marked all nestlings on the back using waterproof marker pens (randomly using red, blue, and green and pairwise combinations of these colors if there were more than three nestlings). We checked survival almost daily (daily on weekdays, occasionally during weekends) until offspring became independent (age 35 days). As a measure of early-growth condition, we determined body mass of each nestling to the nearest 0.1 g at 8 days of age (hereafter, condition). Despite the fact that high-quality food was available to all parents ad lib., nestling body mass at this age ranged from about 1.5 to 12.6 g (mean =  $7.1 \pm 1.7$  SD). For 297 of 6,190 nestlings, body mass was measured on day 6, 7, or 9. For those individuals, we estimated their mass on day 8 as follows. We constructed a linear mixed effects model, with nestling body mass as the dependent variable, actual age of the mass measurement and  $F_{\text{ped}}$  as two continuous covariates, and population (1–4; see above) as a fixed factor. We also included the identity of the genetic mother as a random effect. Using the slope of daily mass gain, we estimated mass at day 8 for those 297 individuals by adding or subtracting 0.97 g per day of measuring too early or too late. Because the four populations differ in body mass, we normalized (Z scaled) all measured or estimated values of mass at day 8 within each population before further analysis.

We report effects of inbreeding, age, and early condition always with a negative sign, such that negative values of greater magnitude reflect stronger detrimental effects of being inbred, old, or poorly fed. This allows us to meta-summarize the results and to directly compare the strength of the focal fixed effects on reproductive performance.

#### *Measures of Life Span and Reproductive Performance Traits*

Table 2 provides an overview of all traits included in this study. To allow direct comparison and easy interpretation of the fixed effects and additive genetic correlations, we scored all traits such that higher positive values reflect better reproductive performance.

Life span was analyzed in the following subset of birds: five generations of birds from the Seewiesen population (referred to as generations P,  $F_1$ – $F_3$ , and  $S_3$ ;  $N = 1,855$  individuals) and four generations of birds from the Bielefeld population ( $F_1$ – $F_4$ ;  $N = 1,067$  individuals). Among those



birds, we used the four most complete generations, P and F<sub>1</sub>–F<sub>3</sub> Seewiesen, for which we recorded the exact life span for all ( $N = 1,175$  individuals) as a pool to impute missing life spans. For 219 S<sub>3</sub> Seewiesen birds and for 663 Bielefeld birds, no date of natural death was available (e.g., because individuals were still alive or because their fate was unknown). For these individuals, we used imputed life expectancy in all analyses, defined as the average life span of individuals from the same pool that lived longer than the focal bird when last observed alive.

In aviaries, we identified social pairs by behavior (clumping, allopreening, and visiting a nest together). All parentage assignments were based on conventional microsatellite genotyping using 10–15 microsatellite markers on up to 13 chromosomes (Wang et al. 2017), following Forstmeier et al. (2007a). We assigned every fertilized egg to its genetic mother ( $N = 11,704$  eggs). When the egg appeared infertile (no visible embryo; Birkhead et al. 2008), we assigned it to the social female that was attending the clutch ( $N = 3,630$  cases). In 36 cases where two females used the same nest to lay eggs, we assigned the unfertilized eggs to the female that laid the most similar eggs (in size and shape), based on eggs that were certainly laid by a given female (e.g., fertilized eggs and eggs in other clutches laid by that female). In cases where birds were not allowed to rear offspring, we quantified female fecundity as the total number of eggs laid by the focal female during the breeding period (see tables S1, S2).

In breeding experiments, we opened all unhatched eggs to check for visible signs of embryo development and classified them as either infertile or embryo mortality. In experiments in which all eggs were incubated artificially for a few days to collect DNA from embryos, we classified eggs as infertile or not but discarded information on embryo viability. Visual inspection of opened eggs has the disadvantage that early embryo mortality may get misclassified as infertility if it occurred before any visible signs of development. Misclassification cannot be avoided entirely, even with more time-consuming examination of eggs, which would be challenging to do for thousands of eggs (Birkhead et al. 2008; Murray et al. 2013). However, genotyping the germinal disk and counting sperm on the perivitelline membranes of 76 freshly laid eggs revealed 22 apparently infertile eggs. Only one of those (5%) had more than 20 sperm on the perivitelline membrane, suggesting early embryo mortality (fig. S2; see also Birkhead and Fletcher 1998). In contrast, among 37 eggs with more than 20 sperm on the perivitelline membrane, 36 (97%) developed diploid tissue. Thus, we expect only a small fraction of misclassification.

In cages, we measured male fertility as a binary trait, that is, whether an egg was fertilized. Because extrapair copulations can be excluded in cages, we only genotyped

all surviving offspring with the same set of microsatellites used in aviaries as confirmation (Wang et al. 2017). In 12 cases, one to five eggs (median: one egg) were fertilized by the previous partner of the female, and those were counted as infertile eggs of the focal male. In aviaries, we assessed for each egg whether it was sired by the social male of the female who laid the egg. We refer to this as male within-pair paternity, a trait that reflects a male's ability to defend his paternity against extrapair males. We also quantified male siring success as the total number of fertilized eggs sired by a focal male. This includes males that remained unpaired (without a social female).

For each fertilized egg that was incubated by the social parents, we recorded whether it hatched (binomial trait for the genetic parents). For each hatched egg that was reared, we recorded whether the nestling survived to independence (day 35; binomial trait for the social parents). We quantified the number of seasonal recruits as the number of genetic offspring that survived to independence within a given breeding season. The number of seasonal recruits was square root transformed to approach normality.

### *Statistical Models*

All mixed effects models were run in R version 4.0.0 (R Core Team 2020), using the R package lme4 version 1.1-23 (Bates et al. 2015). All animal models were run using VCE6 (Neumaier and Groeneveld 1998) because (a) it allows running a 12-trait multivariate animal model that consists of 2,346 individuals with at least one trait value per individual and (b) it has a reasonable running time. To check the consistency of model outputs, we repeated all animal models in the R packages pedigreemm version 0.3-3 (Vazquez et al. 2010; univariate animal models only) and MCMCglmm (Hadfield 2010; univariate and bivariate animal models). All model details, with the supporting data and R scripts, have been deposited in the Open Science Framework (<https://doi.org/10.17605/OSF.IO/TGSZ8>; Pei 2020). Model outputs of all methods are given in the supplemental Excel file (available online). The heritability and additive genetic correlation estimates were highly correlated between methods ( $r > 0.65$ ,  $P < .002$ ). We report the VCE6 estimates, unless otherwise stated. Figure S3 shows the exact range of each focal fixed effect and each performance trait value. Here, we Z transformed all covariates and response variables across populations to allow direct comparison of the effect sizes for inbreeding, age, and condition across all models. The 95% confidence intervals of fixed effects from mixed effects models were calculated using the function glht from the R package multcomp version 1.4-13 while controlling

for multiple testing (Hothorn et al. 2008). Data analysis involved four consecutive steps (fig. 1).

*Step 1: estimation of fixed effects and variance decomposition.* The goal of step 1 was to estimate (a) all fixed effects on reproductive performance and (b) individual repeatability of performance traits (fig. 1). All fixed and random effects of models used in step 1 are listed in tables S3 and S4. In brief, we first fitted all models with a Gaussian error distribution to compare and metasummarize the estimated effect sizes of the fixed effects and to estimate the variance components for the random effects. We used all observations with information on the three fixed effects (age,  $F_{\text{ped}}$ , and early condition of the male, female, and the individual egg if applicable) and included population (fixed effect) and female, male, and pair identity (random effects). We analyzed traits that were measured at either egg, clutch, or season level. As applicable, we fitted as fixed effects the laying sequence of eggs within a clutch, the order of hatching of offspring within a brood, the order of the clutches that were laid by a female over the course of a season, the sex ratio in the aviary, and the duration of the season (table S1). For models of embryo survival, we also controlled for whether the eggs were incubated in a nest that still contained offspring from a previous brood (7% of embryos). For models of nestling survival, we added as fixed effect pair type (pair formed through mate choice or through force pairing; Ihle et al. 2015). For models of egg-based fertility, within-pair paternity, and embryo and nestling survival, we also tested the effect of egg volume on egg fate (we calculated volume as  $V = (1/6) \times \pi \times \text{width}^2 \times \text{length}$ , where egg length and width had been measured to the nearest 0.1 mm). For this analysis, we fitted the mean egg volume of each female and the centered egg volumes (centered within individual females) to distinguish between the effects of between- and within-female variation in egg size (van de Pol and Wright 2009). We estimated the variance components for male, female, and pair identity and further controlled for clutch identity and identity of the setup (see tables S1, S2), as applicable, by adding them as random effects. Life span had no repeated measurement; therefore, we included only individual identity as a dummy random effect for practical reasons when running the model and extracting estimates in R. For this lm model, the correlation between the residuals and the dummy random effect equals 1, and the fixed effect estimates were unaffected by the dummy variable. Table 2 shows for which group of individuals, that is, female, male, or the offspring itself, we tested which focal fixed and random effects.

To allow direct comparison of the magnitude of fixed effects at the same level of measurement, we also aggregated data within clutches (e.g., proportion of infertile eggs within a clutch) and within individuals over the course of a season. Models on aggregated data were weighted by

the number of eggs within a clutch or by the number of eggs or clutches for an individual within a season (fig. 1). As expected, the proportion of variance explained by male, female, and pair identity increased from the egg level to the season level (see “Results”). However, the relative proportions explained by female, male, and pair identity did not change notably. Therefore, we focus on the analyses of fixed effect estimates at the breeding season level.

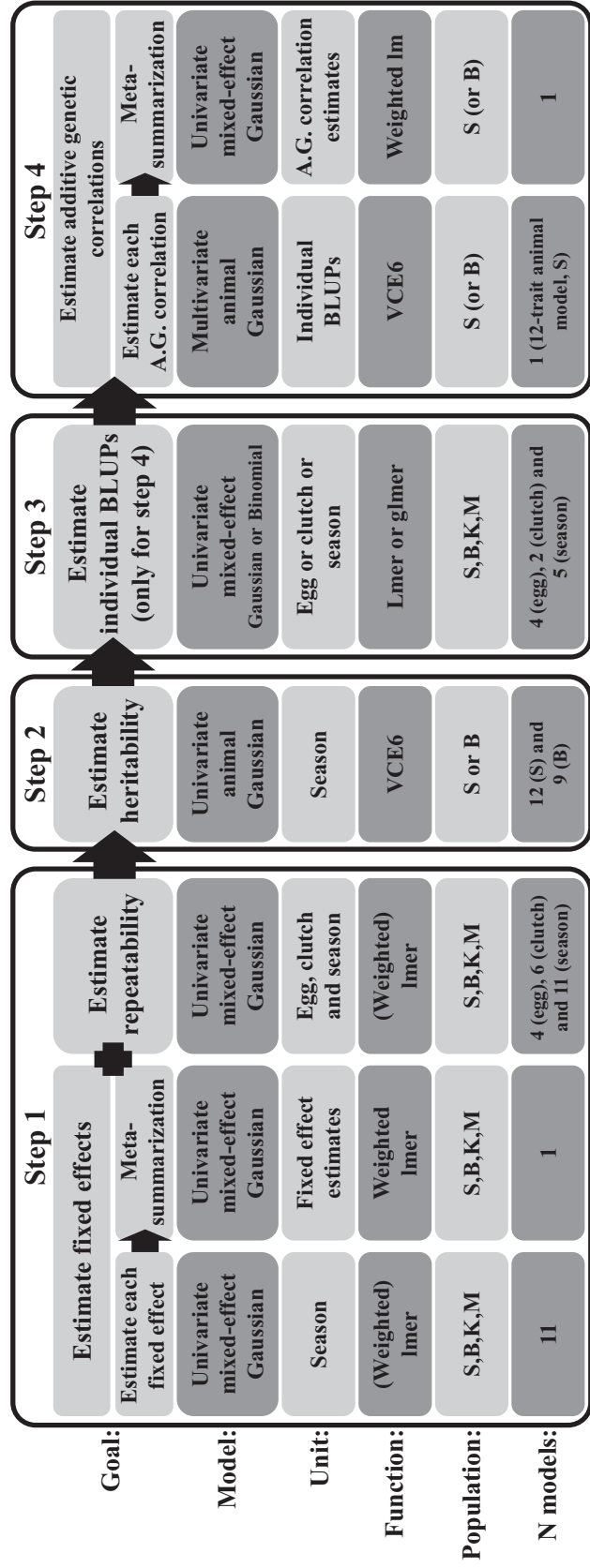
To compare the overall effect sizes between the focal fixed effects, we metasummarized the estimated effect sizes for inbreeding, age, and condition using the weighted lmer function from the R package lme4 (fig. 1, step 1, metasummarization of estimated effect sizes). The uncertainty of each estimate was accounted for by using the multiplicative inverse of the standard error (1/SE) of the response variable as weight. In this metamodel, we used effect size estimates from models that had been aggregated at the season level as the dependent variable. Note that effects of inbreeding of the egg on fertility in cage breeding and nestling survival were taken from egg-based models because they cannot be aggregated by clutch or season. Additionally, we tested whether effect sizes differed among males, females, and offspring (fixed effect with three levels) or among traits (random effect with 11 levels; as listed in table 2).

Additionally, we tested for early-starting aging effects by selecting reproductive performance data for males and females that were <2 years old when reproducing. We then metasummarized the mean age effect estimates using the R function lm, weighted by the multiplicative inverse of the standard error.

We calculated the amount of variance explained by each fixed effect (Nakagawa and Schielzeth 2010) as the sum of squares of the fixed effect divided by the number of observations ( $N - 1$ ; Henderson 1953). In weighted models, we divided the variance components of the fixed effects and the residual by the mean weight value (Bates et al. 2015).

*Step 2: estimation of heritability of fitness-related traits.* The goal of step 2 was to estimate the heritability of reproductive performance traits using univariate Gaussian animal models (fig. 1). Because quantitative genetic models require large amounts of data, we restrict our analyses to the populations Seewiesen and Bielefeld. Note that the pedigrees of our four captive populations are not connected, so it was not useful to analyze them jointly.

We kept the general model structure from step 1 but excluded the fixed effects of egg volume on male fertility, embryo, and offspring survival (to avoid removing biological variation that is potentially heritable and hence of interest; note that the effect sizes of egg volume are small; see “Results”). For the embryo survival model, we excluded the nonsignificant fixed effects of male age, inbreeding,



**Figure 1:** Steps of data analysis from univariate mixed models to multivariate animal models. Shown are the goals of the analysis, the model properties, the unit of analysis (i.e., whether rows in the data represent single eggs, clutches, individuals in a breeding season, single fixed effect estimates, or individuals overall), the software functions used for analysis (for models on aggregated levels, weight stands for the number of eggs or clutches used for each aggregation, whereas in metasummarization models, weight stands for the multiplicative inverse of the standard error of each estimate), and the population abbreviations for data used for the analysis (S = Seewiesen; B = Bielefeld; K = Krakow; M = Melbourne). Shown are the number of models conducted within each step with their specific details (e.g., unit, population, or model type) used for analysis. A.G. = additive genetic.



and condition. For the model on male fertility from cage breeding, we excluded the nonsignificant effect of the level of inbreeding of the egg itself. To most effectively use the available information on reproductive performance, we included individuals with missing values for condition ( $N = 231$  founder individuals and  $N = 23$  individuals of the  $F_2$  generation; i.e., 7% of Seewiesen birds). These missing values were replaced by the population mean. Individual identity was fitted twice, once linked to the individual correlation matrix (pedigree) to estimate the amount of variance from additive genetic effects ( $V_A$ ) and once to estimate the remaining amount of variance from permanent environmental effects ( $V_{PE}$ ; Kruuk and Hadfield 2007). Animal models on nestling mortality were run twice, once for the mother and once for the father. We calculated heritability based on the total phenotypic variance,  $V_{Ph}$ , as  $h^2 = (V_A/V_{Ph})$ , and we also quantified  $V_A$  relative to individual repeatability as  $(V_A/(V_A + V_{PE}))$ .

We compared the estimates of heritability (and  $V_A$  relative to the individual repeatability) between the domesticated population Seewiesen and the recently wild-derived population Bielefeld using the R function lmer. We used the multiplicative inverse of the standard error as weight to control for variation in uncertainty of each estimate. We used the estimates of heritability as the response variable and fitted population as a fixed effect (two levels) and trait as a random effect (nine levels, including only traits that were measured in both populations).

*Step 3: calculation of mean individual fitness-related trait values using best linear unbiased predictions (BLUPs).* The only goal of step 3 was to extract individual estimates of reproductive performance needed for step 4. We kept the model structure from step 1, except that we used a binomial error structure for binary traits, that is, male fertility in cages and aviaries and embryo and nestling survival. Missing values for condition (mostly founders of each population; 6% of all birds of the four populations) were replaced with population means as in step 2. For the embryo survival model, we again excluded the nonsignificant effects of male inbreeding, age, and condition. We also excluded (a) effects of egg volume from all egg-based models and (b) the effect of the level of inbreeding of the egg itself from the model of male fertility measured in cages (see step 2). We extracted the BLUPs for female or male identity (as applicable) as the estimated life-history trait value of that individual (table 2) for step 4.

*Step 4: estimation of additive genetic correlations.* The goal of step 4 was to estimate additive genetic correlations between different performance traits using multivariate animal models. Before fitting a 12-trait animal model that estimates for each matrix (genetic and residual) all 12 variances and 66 covariances simultaneously, we aggregated

the raw data to one phenotypic value per individual for each trait (fig. 1, step 3). This was necessary because we are not aware of software that can handle the full complexity of the underlying raw data (involving more than 26 different fixed effects). Because simple averages of multiple measures can result in outliers when sample size is small, we used the phenotypic BLUPs described above. BLUPs do not produce outliers and account for all considered fixed and random effects (Robinson 1991; Houslay and Wilson 2017). Breeding values (genetic BLUPs) suffer from nonindependence because the phenotype of one individual influences the breeding values of all its relatives (Hadfield et al. 2010). Note that this is not the case for the phenotypic BLUPs we use here. However, the uncertainty that is inherent to each BLUP is not taken into account, which may lead to underestimation of standard errors (Houslay and Wilson 2017). To check the robustness of our results, we compared our estimates with those obtained (a) using a smaller data set from another population (Bielefeld) with the same method and (b) using bivariate animal models in MCMCglmm version 2.29 (Hadfield 2010; population Seewiesen). The latter approach is presumably less powerful than a full 12-trait animal model.

For each of the 12 traits, we fitted an intercept and the pedigree as the only random effect to separate additive genetic from residual variance. We ran these models for the largest and most comprehensive data set (population Seewiesen;  $N = 2,346$  individuals with at least one trait value, BLUPs for 12 traits, and 66 covariances) and for the more limited data set (population Bielefeld;  $N = 1,134$  individuals, BLUPs for 9 traits, and 36 covariances; see “Results”; fig. 1, step 4, estimate additive genetic correlations).

We used the weighted lm function in the R package stats to summarize the estimated additive genetic correlations within and between the major categories of traits, that is, female, male, offspring traits, and life span, for each population separately (table 2; fig. 1, step 4, metasummarization of estimated additive genetic correlations). We fitted the estimates of additive genetic correlations (for each pair of traits, weighted by the multiplicative inverse of the standard error of each estimate) as the dependent variable, with trait class combination as a predictor with seven levels. We removed the intercept to estimate the mean additive genetic correlation for each pairwise combination of classes. We then computed the eigenvectors of the additive genetic variance-covariance matrix of traits, using the R function eigen, and visualized the orientation of the traits in the additive genetic variation space defined by the principle components PC1 and PC2 (Berner 2012). The proportion of variance explained by the first two principle components was calculated using the functions summary and prcomp in the R packages base and stats, respectively.

## Results

### *Effects of Laying and Hatching Order, Clutch Order, and Egg Volume on Egg and Embryo Fate*

The fate of an egg and its embryo depended on the order of laying within a clutch, the order of hatching within a brood, and the order of consecutive clutches within a breeding season (fig. S4; table S3, models at the egg level; see also fig. 1, step 1). First-laid eggs in a clutch were significantly more likely to be infertile or to contain a dead embryo. Fertility and embryo viability were the highest for the third egg (fig. S4). Male fertility significantly increased over the first three clutches and stayed high afterward. In contrast, clutch order did not affect the probability of embryo and nestling survival.

The average effect of egg volume on measures of egg fate was small (mean:  $r = 0.040 \pm 0.016$  SE; fig. S5). Effects of egg volume were largest for nestling survival after hatching and smallest for embryo survival (table S3; fig. S5). Despite large sample size ( $N = 9,785$  eggs), embryo survival was not significantly influenced by egg volume (between-female variation:  $r = 0.015 \pm 0.017$  SE,  $P = .37$ ; within-female variation:  $r = 0.018 \pm 0.010$  SE,  $P = .08$ ; table S3). Additionally, embryos in clutches that were incubated in the presence of nestlings from previous breeding attempts were more likely to die before hatching ( $b = 0.192 \pm 0.048$  SE,  $P < .0001$ ; table S3). Overall, the total amount of variance explained by laying and hatching order, clutch order, and egg volume on egg fate was less than 5% (table S4).

### *Effects of Inbreeding, Age, and Early Condition*

Individuals performed worse in virtually all studied reproductive traits when they were more inbred, as they became older, and when they weighed less at 8 days of age (figs. 2, S3, S6; table S3; see also fig. 1, step 1). Interestingly, reproductive performance did not show an initial increase at a young age (metasummarized effect size of age among birds younger than 2 years:  $r = -0.013 \pm 0.011$  SE; figs. 2C, 2F, 3, A3). Inbred eggs were equally as likely to be infertile as outbred eggs, while inbred embryos and offspring were more likely to die (fig. 3C). Together, this suggests that most infertile eggs were not cases of undetected early embryo mortality. Individuals lived shorter lives when they were inbred and when they had low weight at day 8 (fig. 3; table S3). However, the fixed effects of inbreeding, age, and condition together explained, on average, only 2% of the variance across all traits (fig. 4; table S5).

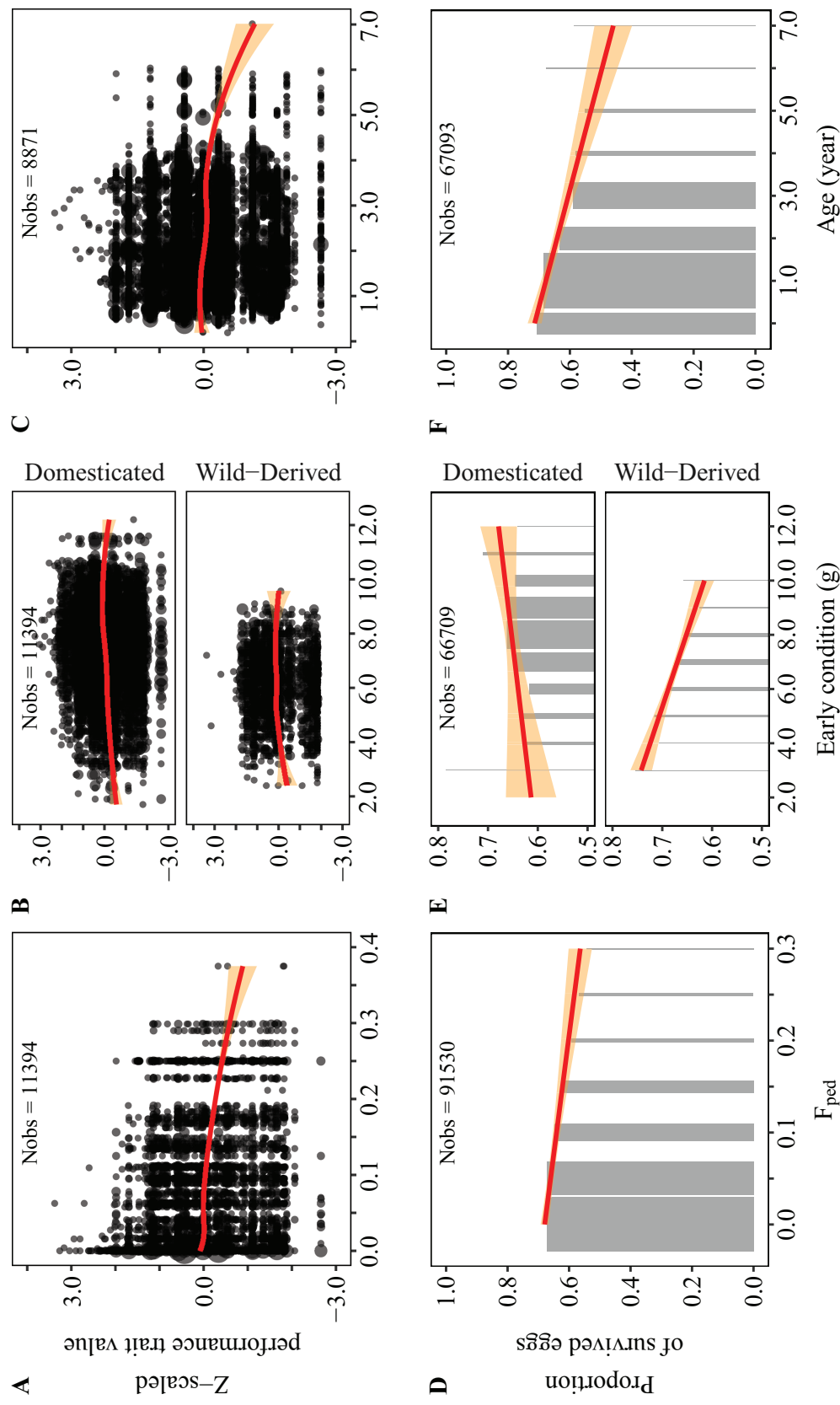
Metasummarized effect sizes of inbreeding ( $r = -0.117 \pm 0.024$  SE) and age ( $r = -0.132 \pm 0.032$  SE) were similar in magnitude and were about twice as large as the remarkably small effect of early condition ( $r =$

$-0.058 \pm 0.029$  SE; fig. 3; table S4; see also fig. 1, step 1, metasummarization of estimated fixed effects). There was no significant difference among males, females, and offspring in how strongly they were affected by these three factors ( $b \leq 0.012 \pm 0.028$  SE,  $P = .63$ ; table S4). Fitting trait (fitness component, 11 levels) as a random effect explained 1.5% of the variance in effect sizes ( $P = .02$ ; table S4), suggesting that some components might be less sensitive than others (fig. 3; table S3). Female traits significantly predicted offspring survival and male fertility (independent of whether they were measured in a cage or in an aviary), whereas male traits showed no effect on offspring survival (fig. 3).

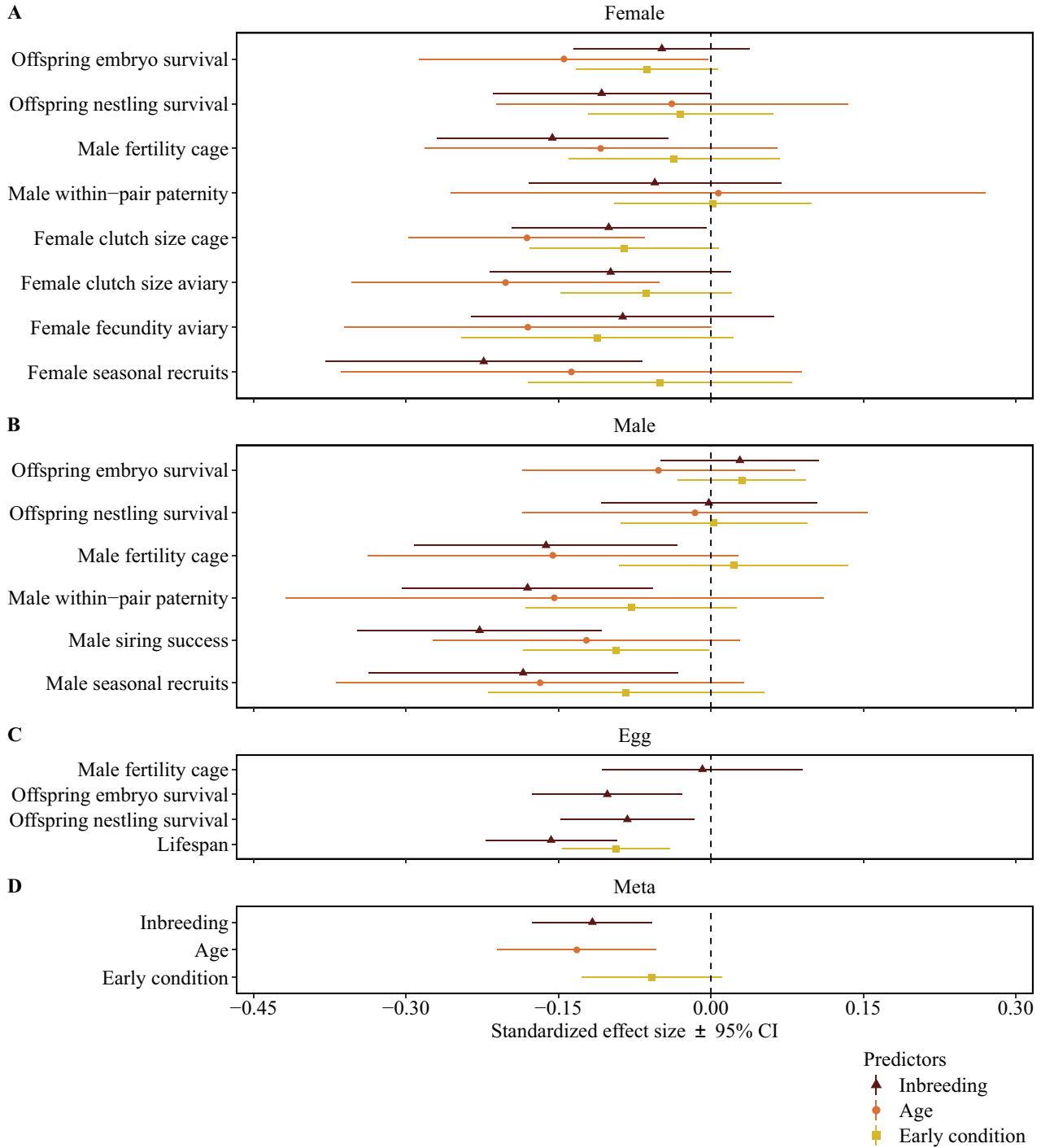
### *Variance Components and Heritability*

Variance components for all reproductive performance traits are shown in figure 4 (see also table S4; fig. 1, step 1, estimate repeatability). Overall, individual reproductive performance traits were significantly repeatable (median  $R = 0.28$ , range: 0.15–0.50). Female reproductive performance traits (clutch size, fecundity, and female seasonal recruits) showed reasonably high repeatability for individual females ( $R \sim 0.26$ –0.40). Likewise, male fertility, male siring success, and male seasonal recruits were highly repeatable for individual males ( $R \sim 0.24$ –0.50). Female reproductive traits from aviary breeding were analyzed independently of whether the focal female had a partner (table 2), but female clutch size measured in a cage showed no contribution from the male partner or from pair identity. In contrast, male fertility depended on all three random effects and was repeatable for males ( $R > 0.23$ ,  $P < .0001$ ) but less so for females ( $R < 0.18$ ,  $P < .1$ ) or for the particular pair combinations ( $R < 0.23$ ,  $P < .05$ ). The model on embryo survival showed significant female and pair identity (genetic parents) effects that were similar in size (both  $R = 0.20$ ,  $P < .0002$ ), while genetic male identity explained no variance (fig. 4). In contrast, social female ( $R = 0.17$ ,  $P = .017$ ) and social male ( $R = 0.15$ ,  $P = .039$ ) identity explained significant amounts of the variance in nestling survival, while the effect of pair identity (parents that raised the brood) was less clear ( $R = 0.14$ ,  $P = .11$ ).

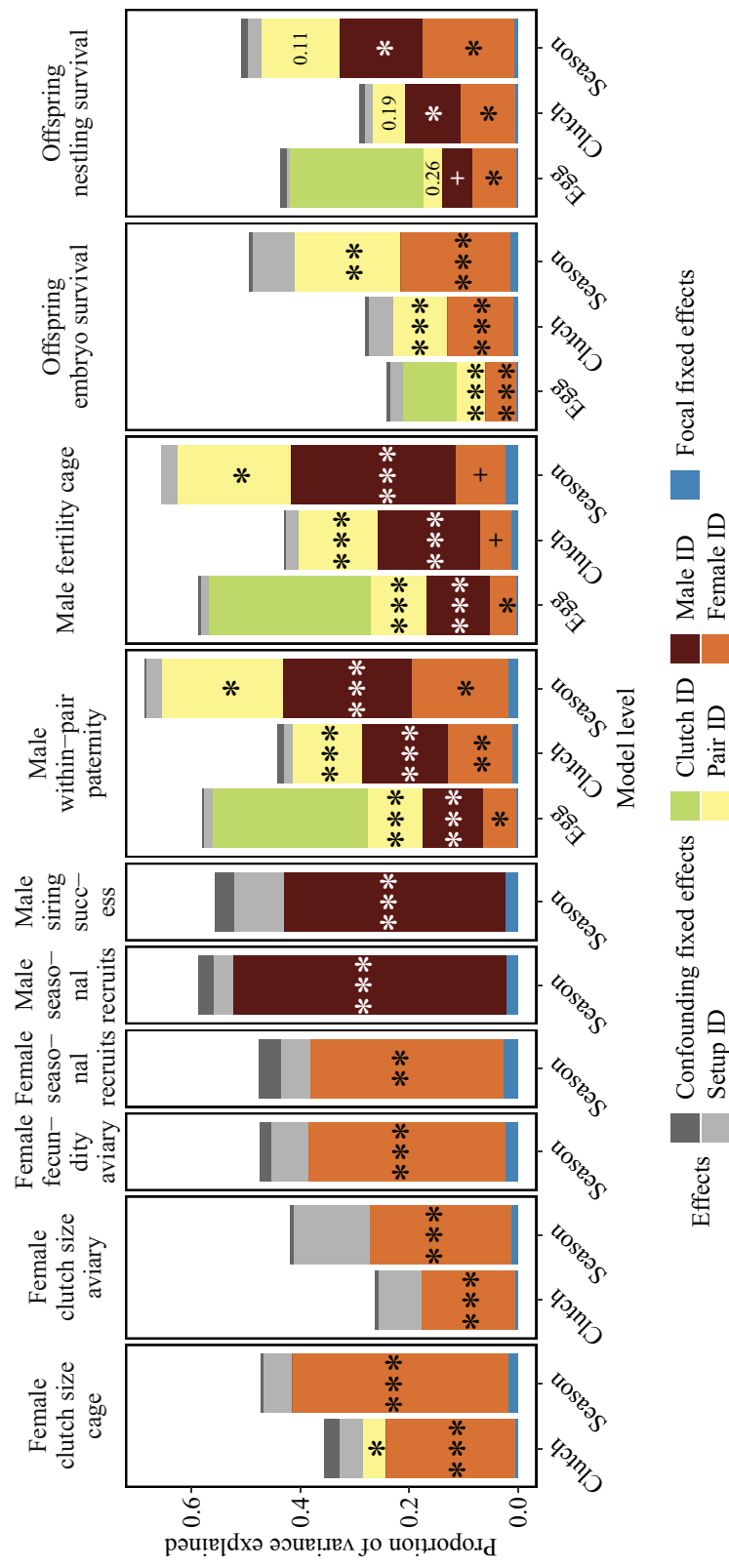
Reproductive performance traits and life span in general had low narrow-sense heritability ( $V_A/V_{Ph}$ ; Seewiesen: median  $h^2 = 0.07$ ; Bielefeld: median  $h^2 = 0.11$ ) and explained only a limited amount of the individual repeatability ( $V_A/(V_A + V_{PE})$ ; Seewiesen: median = 0.29; Bielefeld: median = 0.32; see all heritability estimates in tables S6 and S7; fig. 1, step 2). Heritability estimates from the recently wild-derived population Bielefeld were similar to those from the domesticated Seewiesen population (for nine traits measured in both populations; mean difference in  $h^2 = 0.02$ , range:  $-0.10$  to  $0.13$ , metasummarized



**Figure 2:** Reproductive performance traits (A–C, continuous or count traits; D–F, binomial traits) as a function of inbreeding coefficient ( $F_{ped}$ ; A, D); early condition (mass at day 8), separately for populations that differ in body size (B, E); and age (C, F). Clutch size, fecundity, siring success, seasonal recruits, and life span are continuous or count traits (Z scaled), whereas the proportions of eggs fertilized, embryos survived, and nestlings survived are binomial traits. Note that these are composite figures of all effects that were examined (see fig. S3, available online, for plots of single traits with absolute trait values), such that the fate of one embryo may be shown twice, once as a function of the embryo's own  $F_{ped}$  and once as a function of its mother's  $F_{ped}$  (hence the high sample sizes,  $N_{obs}$ ). The age category zero contains measurements until day 365. Shown are smoothed regressions (red lines) with 95% confidence intervals (orange areas). Circle size (A–C) and bar width (gray; D–F) reflect sample sizes.



**Figure 3:** Standardized effect sizes with their 95% confidence intervals for inbreeding ( $F_{ped}$ ), age, and early condition (mass at day 8) on zebra finch fitness components estimated in univariate Gaussian mixed effects models where all response variables were measured at the level of individuals within seasons and all measurements were Z scaled (table S3, available online). Note that the effect of inbreeding of the offspring on its own mortality was taken from egg-based models. Negative effects of condition indicate low fitness of relatively light-weight individuals at 8 days of age. Panels separate effects of condition, age, and inbreeding of the female (A), the male (B), and the individual egg itself (C). Panel D shows the metasummarized effect sizes for reproductive performance and life span (table S4, available online). The X-axes indicate effect sizes in the form of Pearson correlation coefficients.



**Figure 4:** Variance components estimated in univariate Gaussian mixed effects models (table S5, available online). Each dependent trait is shown in a separate panel. Within panels, the X-axis separates models according to the unit of analysis, based on egg fate (egg), values per clutch (clutch), or values per individual within a breeding season (season). The Y-axis indicates the proportion of variance explained by random effects after accounting for fixed effects. Focal fixed effects refer to the total variance explained by inbreeding, age, and early condition combined. For the key variance components, numbers show nonsignificant *P* values; otherwise,  $^+ P < .1$ ,  $^* P < .05$ ,  $^{**} P < .01$ ,  $^{***} P < .001$ . Note that models of female clutch size aviary, female fecundity aviary, and female seasonal recruits were analyzed without male ID and pair ID and, likewise, male seasonal recruits and male siring success were analyzed without female ID and pair ID because not all birds form a pair bond; male ID explained no variance in models of clutch size cage and embryo survival, while pair ID explained no variance in clutch size cage model.



difference after controlling for the uncertainty of each estimate:  $\Delta b < 0.0001$ ; mean difference in  $V_A/(V_A + V_{PE}) = 0.20$ , range:  $-0.13$  to  $0.68$ , metasummarized difference:  $\Delta b = 0.0002$ ; table S8).

### Additive Genetic Correlations

Reproductive performance traits were grouped into three classes: (1) aspects of male reproductive performance, (2) aspects of female reproductive performance, and (3) aspects of offspring survival (table 2). Traits within each of these classes were, on average, positively correlated with each other at the additive genetic level (for the Seewiesen population, female traits: mean  $r_A = 0.66$ ,  $P < .0001$ ; male traits: mean  $r_A = 0.67$ ,  $P < .0001$ ; offspring survival traits: mean  $r_A = 0.36$ ,  $P = .09$ ; fig. 5A; see also fig. 1, steps 3 and 4). Results for the Bielefeld population are shown in figure S7. The metasummarized results are given in table S9, and all additive genetic correlation estimates are listed in tables S10 and S11 (fig. 1, step 4). Estimates of the additive genetic correlations from bivariate animal models using MCMCglmm are shown in figures S8 (Seewiesen) and S9 (Bielefeld).

Male and female reproductive performance traits were weakly negatively correlated at the additive genetic level (mean  $r_A = -0.14$ ,  $P = .04$ ; see MF in figs. 5A, S8A). Accordingly, the eigenvectors for male and female fitness traits were pointing in different directions (figs. 5B, S8B). This pattern was somewhat consistent between the Seewiesen and Bielefeld populations (see figs. S7 and S9 for the Bielefeld population). However, the negative correlation between male and female fitness traits was no longer significant when estimated by the bivariate animal models in MCMCglmm and disappeared in the Bielefeld data set (table S9). The orientation of offspring survival traits relative to male and female fitness traits was less consistent. In the Seewiesen population, female fitness traits were positively correlated with offspring survival traits at the additive genetic level (mean  $r_A = 0.61$ ,  $P < .0001$ ), while male fitness traits were not aligned with offspring survival traits (mean  $r_A = -0.11$ ,  $P = .24$ ; fig. 5). In contrast, in the Bielefeld population, both female and male fitness traits were positively correlated with offspring survival traits (fig. S7). Life span tended to be positively correlated with all reproductive performance traits (Seewiesen: mean  $r_A = 0.19$ ,  $P = .02$ ; Bielefeld: mean  $r_A = 0.60$ ,  $P = .0006$ ; figs. 5, S7; table S9).

## Discussion

### Effects of Inbreeding, Age, and Early Condition

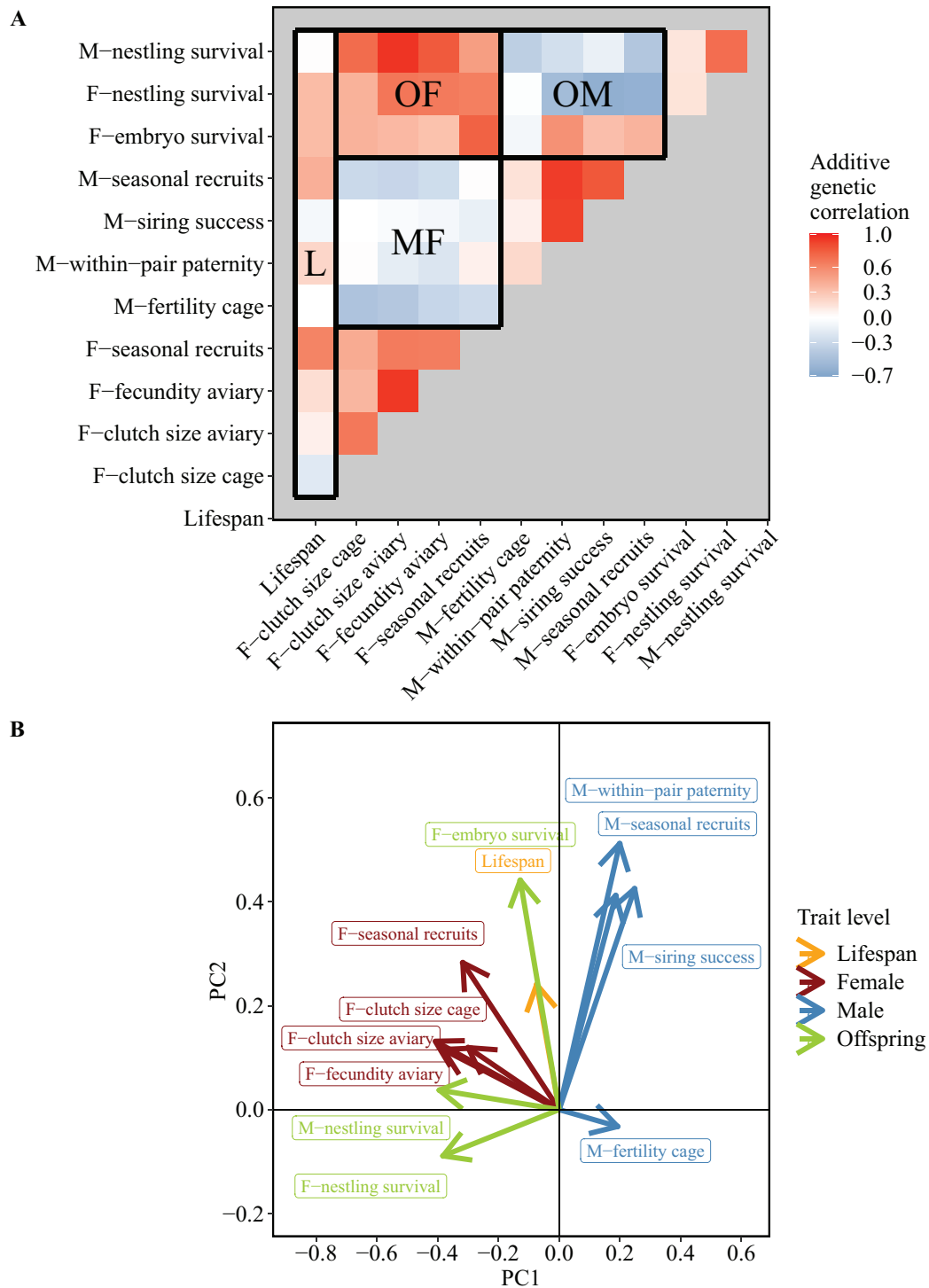
Many studies have shown that inbreeding depression significantly influences morphological, behavioral, and fitness-

related traits in zebra finches (Bolund et al. 2010a; Forstmeier et al. 2012; Hemmings et al. 2012; Opatová et al. 2016) and in other species (Amos et al. 2001; Reed and Frankham 2003; Williams et al. 2003; Michaelides et al. 2016). This study confirms that inbreeding negatively influenced all phases of offspring survival, reproductive performance, and life span. We found that the level of inbreeding of both genetic parents negatively influenced egg fertility, suggesting that this is a matter of not only sperm functionality (Opatová et al. 2016) but also female reproductive performance (e.g., egg quality or copulation behavior). Male and female fitness estimates (seasonal recruits) were most strongly affected by inbreeding (fig. 3), presumably because the successful rearing of offspring to independence requires proper functionality at every step of reproduction.

Age effects on reproductive performance typically show an initial increase in performance in both short- and long-lived species (e.g., in great tits *Parus major* [Bouwhuis et al. 2009], wandering albatrosses *Diomedea exulans* [Lecomte et al. 2010], Houbara bustards *Chlamydotis undulata* [Preston et al. 2011], Langur monkeys *Presbytis entellus* [Harely 1990], and red deer *Cervus elaphus* [Pemberton et al. 2009]). Interestingly, in our captive zebra finches we found that reproductive performance (especially male fertility, female clutch size, fecundity, and female effects on embryo survival) did not show an initial increase after birds reached sexual maturity at about 100 days of age (figs. 2C, 2F, A3). This could be because zebra finches are short-lived opportunistic breeders that reach sexual maturity earlier compared to most other birds (Zann 1996). Thus, zebra finches might have been selected to perform best early on. Alternatively, this effect may not be present in the wild, where experience might play a more important role in determining reproductive success.

Over the past decades, numerous studies focused on how early developmental conditions affect behavior, life history, and reproductive performance later in life (Tschirren et al. 2009; Rickard et al. 2010; Boersma et al. 2014). Here we show that even dramatic differences in early growth conditions of surviving offspring (see range of X-axis in fig. 2B) have remarkably small (though statistically significant) effects on adult reproductive performance.

Overall, the proportion of variance explained by inbreeding, age, and early condition (characteristics of conditions) was less than 3% (fig. 4; table S4). This indicates that individuals' robustness against poor conditions appears more noteworthy than their sensitivity. As will be discussed below, the majority of the individual repeatability in reproductive performance cannot be explained by such individual characteristics.



**Figure 5: G matrix of reproductive performance traits and life span estimated from a multivariate animal model for the Seewiesen population (shown are estimates from VCE6; for estimates of MCMCglmm bivariate models, see fig. S8; see also figs. S7 and S9 for estimates from the Bielefeld population; estimates are given in tables S10 and S11; all are available online). A, Heat map of additive genetic correlations between components of male (M), female (F), and offspring (O) fitness and life span (L). Red indicates a positive genetic correlation between traits, while blue indicates a negative correlation. Blocks marked in bold emphasize correlations between categories (e.g., MF stands for correlations between male and female fitness components). B, First two principal components of the G matrix, showing eigenvectors of the 12 fitness components. The amount of variance explained by PC1 and PC2 is 67% and 8%, respectively. Note that aspects of male fitness do not align with aspects of female and offspring fitness.**

*Repeatability and Heritability  
of Reproductive Performance*

Individual zebra finches were remarkably repeatable in their reproductive performance. Our variance-partitioning analysis showed that infertility is largely a male-specific trait, whereas embryo and offspring survival are mostly related to female identity (fig. 4; table S4). In contrast, in polyandrous crickets, egg hatching (primarily a matter of embryo survival) was mostly influenced by male identity (García-González and Simmons 2005; Ivy 2007). The effects of pair identity on infertility and offspring mortality in zebra finches may reflect behavioral incompatibility, while the pair effect on embryo mortality more likely reflects genetic incompatibility (Ihle et al. 2015).

Although male and female zebra finches are highly repeatable in their reproductive performance, the heritability of fitness traits was low. Heritability estimates were similar between the recently wild-derived Bielefeld population and the domesticated Seewiesen population. This contradicts the idea that ongoing adaptation to captivity would result in a higher heritability of fitness traits. Overall, our findings indicate that there are some additive genetic components underlying zebra finch reproductive performance.

*Evidence for Sexually Antagonistic Pleiotropy and  
Other Potential Causes of Reproductive Failure*

Some of the standing additive genetic variance in reproductive performance could be maintained by intralocus sexual antagonism between male fitness traits and female (and offspring) fitness traits (Cox and Calsbeek 2009). This has, for example, been suggested in quantitative genetic studies on *Drosophila* (Innocenti and Morrow 2010), red deer (Foerster et al. 2007), and the bank vole *Myodes glareolus* (Mills et al. 2012). We found that male fertility, siring success, and seasonal recruitment were overall negatively correlated with female fitness and offspring survival traits, suggesting that alleles that increase male fitness tend to reduce female and offspring fitness (fig. 5). In contrast, life span and reproductive performance tended to be positively correlated at the additive genetic level, which is suggestive of some overall good gene variation in our population (fig. 5). Some words of caution should be added to these observations. VCE6 (figs. 5, S7) yielded higher absolute values of estimates than those calculated with the R functions PedigreeMM (heritability estimates only) and MCMCglmm (see figs. S8, S9; also see tables S6, S7, S10, S11). Nevertheless, the additive genetic correlation estimates are highly correlated between the two methods ( $r > 0.7$ ,  $P < .0001$ ; see tables S10, S11). Estimating genetic correlations between traits with low heritability requires large data sets, especially on additive genetic correlations of between-sex reproductive performance

where the traits of male fertility and female clutch size in cages are missing ( $N$  performance traits: Seewiesen = 12, Bielefeld = 9;  $N$  birds have at least one entry of reproductive performance data: Seewiesen = 2,346, Bielefeld = 1,134; hence, these results are presented in fig. S7). Despite this lack of power in our second-largest data set of population Bielefeld, its overall orientation of traits in the additive genetic variation space of the principle components PC1 and PC2 is very similar to population Seewiesen (note that life span is in the center of all fitness traits and that aspects of female fitness do not align with male fitness in figs. 5B, S7B, S8B, and S9B).

Individual repeatability of fitness-related traits could arise from permanent environmental effects (e.g., early developmental conditions and long-lasting diseases) or from genetic effects. However, although food shortage experienced during early development (reflected in body mass at 8 days old) strongly predicted nestling mortality (Pei et al. 2020), it explained only <1% of variation in reproductive performance later in life (mean  $r = -0.058$ ; figs. 2B, 2E, 3, 4). Additionally, our captive zebra finches were raised and kept in a controlled environment with no obvious diseases detected. Additive genetic effects explained only about 30% of the large remaining unexplained individual repeatability in fitness-related traits, suggesting that reproductive performance might be (predominantly) dependent on genetic effects of local over- or underdominance and epistasis, that is, incompatibility between loci. For instance, high levels of reproductive failure could be maintained when alleles show nonadditive effects, with selection favoring the heterozygous genotype (see, e.g., Sims et al. 1984; Grossen et al. 2012). In zebra finches, males that are heterozygous for the inversion on the Z chromosome produced fast-swimming sperm and sired more offspring (Kim et al. 2017; Knief et al. 2017), while heterozygous males for inversions on chromosomes Z and 13 produced slightly more dead embryos, likely caused by unbalanced crossover during spermatogenesis (Knief et al. 2016a). However, these phenomena explain only a small fraction of infertility and embryo mortality. Overall, there is little over- or underdominance for fitness related to the major inversion polymorphisms that segregate in wild and captive zebra finch populations (Knief et al. 2016a).

Epistatic effects that involve several genes (e.g., incompatibility between nuclear loci or between mitochondrial and nuclear genomes; Zeh and Zeh 2005) could be evolutionarily stable when certain combinations of genotypes perform better than others, especially when combined with overdominance. Examples of incompatibilities are mostly known from hybrid systems (Arntzen et al. 2009; Hermansen et al. 2014; Eroukhanoff et al. 2016), but they could also be segregating within a species after the

mixing of two lineages that have evolved weak incompatibilities. Some studies on inbred lines in invertebrates found evidence of mitonuclear incompatibilities. For example, in the spider mite *Tetranychus evansi*, the eggs of F<sub>1</sub> hybrid females of two genetic lineages showed higher hatching failure compared to the pure parental lines (Knecht et al. 2017), and in *Drosophila melanogaster*, the interaction of mitonuclear background explained a small but significant amount of variation in female fitness (Downing et al. 2007).

Infertility, as one of the main and puzzling sources of reproductive failure, behaved as a male-specific trait that may also depend in part on behavioral compatibility between pair members (reflected in copulation behavior) and in part on the male's genotype at sexually antagonistic loci. The intrinsic male fertility, measured in a cage, that is, in the absence of sperm competition, correlated negatively with all female and offspring survival traits at the additive genetic level (sexual antagonism; median  $r_A = -0.30$ , range:  $-0.45$  to  $-0.01$ ; fig. 5; table S10). In contrast, in the presence of sperm competition (aviary breeding), high male within-pair paternity, siring success, and seasonal recruitment should also be influenced by the competitive ability of the individual, and this could explain why these traits correlated positively with life span and trade off less with female traits and offspring rearing ability at the additive genetic level (figs. 4, S7; tables S10, S11).

Embryo mortality, another main source of reproductive wastage, mostly depended on the identity of the genetic mother and the identity of the genetic pair members. A previous study using cross fostering of freshly laid eggs also showed that embryo mortality is a matter of the genetic parents rather than the foster environment (Ihle et al. 2015). The female component suggested an overall female genetic quality effect yet with limited heritability (pointing toward dominance variance or epistasis). The effect of the combination of parents on embryo mortality might reflect an effect of the genotype of the embryo itself, possibly involving multilocus incompatibilities (Corbett-Detig et al. 2013).

### Conclusions

Our results suggest that sexually antagonistic pleiotropy between male and female fitness plus offspring rearing traits may maintain some of the existing additive genetic variation in reproductive performance traits in captive zebra finches. Additionally, there appears to be some “good gene” (heritable) variation among reproductive performance traits and individual life span, which suggests an ongoing adaptation to the captive environment. We found that the level of inbreeding, age, and—to a lesser extent—early rearing conditions predicted a small but

statistically significant amount of variation in individual reproductive performance and life span. However, those three effects were so small that they cannot be the main causes of reproductive failure. Our results show that fertility is mostly influenced by the male, whereas embryo and nestling survival are mainly influenced by the female. Although individual zebra finches were moderately repeatable in their reproductive performance, the heritability of those traits was low. Overall, our results suggest that alleles that have additive effects on fitness might be maintained through sexually antagonistic pleiotropy and that the major genetic causes of reproductive failure might be determined by genetic incompatibilities or local dominance effects.

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### Statement of Authorship

W.F. and B.K. designed and planned the study. W.F., D.W., and K.M. collected reproductive performance data. Y.P. and W.F. analyzed the data, with input from J.R. Y.P., W.F., and B.K. interpreted the results and wrote the manuscript, with input from J.R. All authors contributed to the final manuscript.

### Data and Code Availability

Supporting data, model structures, and R scripts have been deposited in the Open Science Framework (<https://doi.org/10.17605/OSF.IO/TGSZ8>; Pei et al. 2020).

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A family of zebra finches (*Taeniopygia guttata*). The mother (left) is feeding one of the three fledglings while the father (right) is watching the surrounding. Drawing by Yifan Pei.